REMARKS

Claims 1, 14, and 19 have been amended to recite "identifying a patient having refractory depression characterized by dissociation." Support for the claim amendments is found, for example, in paragraphs 30-40, pages 11-14 of the specification. Claims 1, 14, and 19 have also been amended to recite "a kappa receptor opiate antagonist or an opiate antagonist having kappa receptor antagonist activity." Support for these claims amendments is found, for example, in paragraph 51 of the specification with regard to kappa receptor opiate antagonists and paragraph 44 of the specification with regard to opiate antagonists having kappa receptor antagonist activity. Paragraph 44 discusses nalmefene as "the preferred opiate antagonist." Nalmafene is a well-known opiate antagonist having kappa receptor antagonist activity. See *Binding of a New Opiate Antagonist, Nalmefene, to Rat Brain Membranes*, Mary Ellen Michel et al., Meth and Find Exptl Clin Pharmacol 1985; 7(4): pgs. 175-177. The amendments do not introduce any new matter.

35 U.S.C. § 103(a)

Claims 1-23 remain rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Dante ('091) and Carlezon Jr. ('075). According to the office action, "Dante teaches use of opiate antagonists (columns 2-3) along with typical anti-depressants for the treatment of refractory depression as well as depression with anxiety" and Carlezon Jr. teaches the use of Kappa receptor antagonists for treatment of major depression and *post traumatic stress disorder*, from which the dissociative symptoms appear." Office Action at 2. The Applicant respectfully traverses this rejection.

Dante fails to teach or suggest identifying patients with refractory depression characterized by dissociation (i.e., the subset of patients to be treated with opiate

antagnonists and anti-depressants). The claims, as amended, recite "identifying a patient having refractory depression characterized by dissociation." Dante refers to several categories of depression and anxiety (e.g., obsessiveness, depression with obsessiveness, depression with anxiety, mania, depression associated with biopolar conditions such as manic depression, depression with manic episodes, and depression concomitant with an illness causing seizures). '091 patent, col. 2, l. 64 – col. 3, l. 4. However, depression characterized by dissociation is noticeably absent. None of the patients described in Dante exhibit refractory depression characterized by dissociation. Thus, Dante utterly fails to teach or suggest identification or treatment of patients with refractory depression characterized by dissociation.

Carlezon Jr. is not prior art with respect to this application. Carlezon Jr. (now U.S. Patent Number 6,528,518), issued on March 4, 2003 from U.S. Patent Application No. 10/027,135, filed on December 20, 2001. The instant application was filed on August 9, 2001. Therefore, Carlezon Jr. cannot be used to reject Applicant's claims. Furthermore, Carlezon Jr. fails to mention refractory depression characterized by dissociation. Carlezon Jr.'s data regarding the effects of kappa receptor antagonists is directed entirely to depression in animal models. Depression characterized by dissociation cannot be diagnosed in animals. Thus, Carlezon Jr. provides no motivation whatsoever for using kappa receptor antagonists in combination with antidepressants for the treatment of depression characterized by dissociation in humans.

A proper rejection under 35 U.S.C. § 103(a) requires that the cited references teach or suggest all the claims limitations. MPEP § 706.02(j)("the prior art reference (or references when combined) must teach or suggest all the claim limitations)." Dante fails to teach or suggest all the elements of the claims and Carlezon Jr. is not prior art and fails to teach or suggest all the elements of the claims. Therefore, this rejection under 35 U.S.C. § 103(a) should therefore be withdrawn.

Claims 1-23 stand rejected under 35 U.S.C. § 103(a) as allegedly being obvious in view of Glover '612. According to the office action, Glover refers to an example (Example 2) of a patient "who became depressed while on the maltrexone for symptoms of dissociation" and received a tricyclic antidepressant "with a very positive response." There is no indication anywhere in Glover that the patient suffered from refractory depression. With regard to the patient of Example 2, the '612 patent states that the tricyclic antidepressant "would not have helped her during the numb state." '612 patent, col. 9, ll. 21-25. In fact, the patient was "no longer numb" after treatment with opiate antagonist before she exhibited signs of depression. Id. at ll. 15-25. Thus, the patient of Example 2 never exhibited refractory depression characterized by dissociation. Rather, she exhibited dissociation followed by depression. Thus, Glover '612 does not teach identifying or treating patients having refractory depression characterized by dissociation.

Furthermore, Glover '612 teaches away from the claimed invention by stating that the combination of opiate antagonist and tricyclic antidepressant would not have alleviated the patient's symptoms during the numb state. Col. 9, ll. 21-24. Based on Glover '612, there is no reasonable expectation that the combination of opiate antagonist and an anti-depressant could be used to treat a patient suffering from refractory depression characterized by dissociation. This rejection under 35 U.S.C. § 103(a) should therefore be withdrawn.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If the Examiner should believe that anything further may be required to place this application in even better form for allowance, she is cordially invited to telephone the undersigned attorneys for the Applicant.

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Respectfully submitted,

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Binding of a New Opiate Antagonist, Nalmefene, to Rat Brain Membranes

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SUMMARY

Nalmefene (6-methylene-naltrexone) is a potent, orally active, opiate antagonist. IC_{50}'s were obtained for nalmefene, naloxone and naltrexone using radiolabelled prototype ligands for μ, κ and δ receptors in homogenates of rat brain minus cerebellum. Nalmefene antagonized the bindings of [³H]-dihydromorphine, [³H]-ethylketocyclazocine and [³H]-D-ala-D-leu enkephalin with IC_{50}'s in the low nanomolar range. At the central μ receptor, nalmefene bound with an IC_{50} of 1.0 nM, equal to that of naltrexone and approximately four times lower than that of naloxone. At central κ and δ sites the IC_{50}'s for nalmefene were somewhat lower than those of naltrexone and considerably lower than those of naloxone. All three antagonists had sodium indices < 1.0. These results indicate that nalmefene is a universal opiate antagonist, has no agonist character at the central μ site and binds more effectively to central opiate receptors than either naloxone or naltrexone.

Key words: Opiates - Nalmefene - Opiate antagonist

INTRODUCTION

Nalmefene is the generic name of the compound 6-desoxy-6-methylene-naltrexone (1). In the mouse hot plate and tail clip procedures, this compound is much more potent than naloxone or naltrexone in blocking the analgesia produced by an ED_{95} dose of morphine (1). Nalmefene showed similar improved potency and duration of action over naloxone and naltrexone when tested against the agonist 6-azidomorphine in the isolated guinea pig ileum (2). In morphine-dependent rats nalmefene, naloxone and naltrexone increased tail skin temperature (TST), an indication of opiate antagonism. Determination of ED_{50}'s for the TST response revealed the expected relative potency for the narcotic antagonists: nalmefene > naltrexone > naloxone (3).

In order to help explain this increase in potency, we wished to test the binding of nalmefene to μ, κ and δ opiate receptors. We chose to compare the displacement of radiolabelled prototype ligands by nalmefene, naloxone and naltrexone in homogenates of rat brain.

MATERIALS AND METHODS

Tissue

Whole rat brains (minus cerebella and cleaned of meninges) were homogenized via Polytron in 20 volumes of 20 mM sodium-free HEPES buffer, pH 7.4 and pelleted by centrifugation at 15,000 rpm for 15 min. The tissue was resuspended in 40 vol. HEPES and incubated in a 37°C shaking water bath for 30 min to eliminate endogenous opiate activity. After another centrifugation the tissue pellet was resuspended in 60 vol. HEPES. This protein solution was used in the assay.

Ligands

The following [³H]-ligands (New England Nuclear) were used: dihydromorphine (DHM), specific activity 80.3 Ci/mM, assay concentration 1 nM; ethylketocyclazocine (EKC), specific activity 16.4 Ci/mM, assay concentration 4 nM; D-ala-D-leu enkephalin (DADLE), specific activity 43.6 Ci/mM, assay concentration 3 nM.

0379-0355/85/$5.00 + $2.00

Each ligand was run in three separate homogenates of rat brain.

Assay

The total assay volume was 2.0 ml; all tubes were run in duplicate and were arranged as follows:

Total (T)
- 0.9 ml HEPES (sodium-free)
- 0.1 ml [^{3}H]-ligand
- 1.0 ml tissue

Non-specific (NS)
- 0.8 ml HEPES
- 0.1 ml [^{3}H]-ligand
- 0.1 ml unlabelled ligand (2×10^{-5} M)
- 1.0 ml tissue

Nalmefene, naloxone and naltrexone were diluted serially from 10^{-11} to 10^{-6} M.
- 0.8 ml HEPES
- 0.1 ml [^{3}H]-ligand
- 0.1 ml drug dilution
- 1.0 ml tissue

Sodium shift: 0.2 ml 100 mM NaCl was added to all tubes and buffer reduced to 0.6 ml; all other additions were as above.

All tubes were kept on ice until after addition of protein, then incubated in a shaking water bath at 25°C for 30 min. Assays were filtered on a Brandel Cell Harvester through GF/B (Whatman) filter paper and washed (2×5 ml) with cold HEPES. Filters plus 8 ml scintillation cocktail were counted overnight and CPM's converted to % inhibition:

$$100 - \left(\frac{\text{drug}_{cpm} - \text{NS}_{cpm}}{\text{T}_{cpm} - \text{NS}_{cpm}} \right)$$

The results of three experiments were averaged and plotted on 4 cycle semilog paper to obtain dose response curves and IC_{50}'s for the three antagonists.

RESULTS

Nalmefene displaced the binding of [^{3}H]-DHM, [^{3}H]-EKC and [^{3}H]-DADLE with IC_{50}'s in the nanomolar range. A comparison of IC_{50}'s for nalmefene, naloxone and naltrexone may be found in Table 1. At the central μ receptor nalmefene showed equal potency with naltrexone and was approximately four times more potent than naloxone. Nalmefene was the most potent of the three antagonists at central κ and δ receptors, especially when compared to naloxone. The relative

TABLE 1. IC_{50}'s (nM) of 3 opiate antagonists in binding to rat brain membranes

	[^{3}H]-DHM	[^{3}H]-EKC	[^{3}H]-DADLE
Nalmefene	1.0	5.1	6.1
Naloxone	3.5	60	26
Naltrexone	0.9	10	10

binding of these three antagonists to central μ, κ and δ receptors may be represented by the ratio

$$\frac{IC_{50}\ X}{IC_{50}\ \text{naloxone}};$$

values appear in Table 2.

TABLE 2. Relative binding of naloxone, naltrexone and nalmefene at central opiate receptors

	Naloxone	Naltrexone	Nalmefene
μ	1.0	0.26	0.29
κ	1.0	0.17	0.09
δ	1.0	0.38	0.23

Addition of NaCl to the assay buffer increased the binding at the μ receptor for all three antagonists. Sodium ratios were obtained:

$$\frac{IC_{50}\ \text{with NaCl}}{IC_{50}\ \text{without NaCl}}$$

and are summarized in Table 3. All three antagonists had a sodium index < 1.0.

TABLE 3. Sodium ratios for nalmefene, naloxone and naltrexone binding at the central μ receptor

Nalmefene	0.73
Naloxone	0.45
Naltrexone	0.60

DISCUSSION

The increased potency of nalmefene, compared to naloxone and naltrexone, is supported by binding data from rat brain membranes. Of particular interest is the affinity of nalmefene for the kappa and delta

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Naloxone is known to displace mu ligands more effectively than it displaces kappa or delta ligands (4). Both naltrexone and nalmefene showed improved binding at these two sites; however, nalmefene was the most effective of the three antagonists. Since guinea pig ileum contains a substantial kappa population (5, 6), the potency of nalmefene in this preparation could be due to its binding characteristics.

Nalmefene, naloxone and naltrexone all had sodium indices less than 1.0, an indication of pure opiate antagonists (7). Nalmefene, then, should have no abuse potential and could be used safely in longterm treatment requiring opiate antagonism. The increased potency of nalmefene may make its use preferable over both naloxone and naltrexone since lower doses would be required.

In summary, nalmefene proved a more effective opiate antagonist than either naloxone or naltrexone. This increased potency was evident at central μ, κ and δ receptors. Furthermore, nalmefene appears to be a pure antagonist.

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